REMARKS

1. Formal Matters

a. Status of the Claims

Claims 21-49 are pending in this application. Claim 35 is amended and claims 50 and 51 are new. Applicant respectfully requests entry of the amendments and remarks made herein into the file history of the present application. Upon entry of these amendments, claims 21-51 are pending and under active consideration.

b. Amendments to the Claims

Claim 35 is amended to correct a typographical error. Support for new claims 50 and 51 can be found at the sequence listing as originally filed.

2. Patentability Remarks

The claims are drawn to isolated viral nucleic acids related to miRNAs, which are short single-stranded RNA molecules that regulate gene expression.[1] MiRNAs regulate gene expression by base pairing with a less than perfect complementary binding site sequence in a target mRNA molecule, which leads to degradation of the target mRNA or silencing of expression of the encoded protein.[2]

Genes encoding miRNAs are transcribed from the genome into RNA precursors of about 50-120 nucleotides in length that form stem-loop structures or "hairpins."[3] The hairpins are characterized in part by stem segments that are typically less than totally complementary. Claims 35-51 are drawn to such hairpin precursors. The hairpin precursors are processed by enzymes such as Dicer to mature miRNAs of about 18-24 nucleotides in length.[4] Claims 21-34 are drawn to such miRNAs.

a. Claim Objections

On page 3 of the Office Action, the Examiner objects to claims 36-49 under 37 C.F.R. § 1.75 as allegedly being a substantial duplicate of claims 21-35 respectively. For purposes of this reply, Applicant believes that the Examiner intended to object to claims 35-49

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[1] *See* page 10, lines 2-9 and 22-26 of the specification, as originally filed.

[2] *See* page 11, lines 1-4 and 12-15 of the specification, as originally filed.

[3] *See* page 4, lines 30-33, page 5, lines 1-6, and page 10, lines 14-19 of the specification, as originally filed.

[4] *See* page 4, lines 30-33, and page 10, lines 21-26 of the specification, as originally filed.

not claims 36-49. The Examiner asserts that the two groups of claims are identical because they both recite a hairpin consisting of 50-131 nucleotides and a nucleic acid of 17-24 nucleotides capable of binding to a binding site of a mRNA.

Applicant respectfully submits that independent claims 21 and 35 are not substantial duplicates because of the differences in limitation (a). As discussed above, claims 21-34 are drawn to miRNAs whereas claims 35-49 are drawn to hairpin precursors. This distinction in the claimed subject matter is apparent after reading the preamble of independent claims 21 and 35 in combination with limitation (a), which recites a length limitation.

The preamble of claims 21 and 35 each indicate that the claims are drawn to a "first viral nucleic acid." For claim 21, limitation (a) recites that "the first viral nucleic acid consists of **17-24** nucleotides." Claim 21 and dependent claims 22-34 are therefore drawn to a viral nucleic acid of 17-24 nucleotides (*i.e.*, a miRNA).

Turning to claim 35, limitation (a) recites that "the first viral nucleic acid consists of **50-131** nucleotides." Claim 35 and dependent claims 36-51 are therefore drawn to a viral nucleic acid of 50-131 nucleotides (*i.e.*, a hairpin precursor).

Although the two groups of claims recite similar features, a careful reading of the preamble in combination with limitation (a) clearly indicates that claims 21-34 and 35-51 are drawn to distinct subject matter. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw the objection to claims 35-49.

b. 35 U.S.C. § 103

On pages 7-15 of the Office Action, the Examiner rejects claims 21-49 under 35 U.S.C. §103(a) as allegedly being obvious over Moss *et al., Current Biology* 12:R138-R140 (2002) (hereafter "Moss"), in view of Yu *et al., J. of Virology* 73:3638-3648 (1999) (hereafter "Yu") and Konings *et al., J. of Virology* 66:632-640 (1992) (hereafter "Konings"). The Examiner further rejects claims 21-49 under 35 U.S.C. §103(a) as allegedly being obvious over Grad *et al., Molecular Cell* 11:1253-1263 (2003) (hereafter "Grad") and Lagos-Quintana *et al., Science* 294:853-858 (2001) (hereafter "Lagos") in view of Konings.

The Examiner asserts that the claims drawn to hairpin precursors are obvious because one of ordinary skill in the art would have been motivated to use the algorithms and computational methods of Moss and Konings to identify viral miRNA hairpin precursors from the viral fold-back structures of Yu. The Examiner also asserts that it would have been obvious to one of

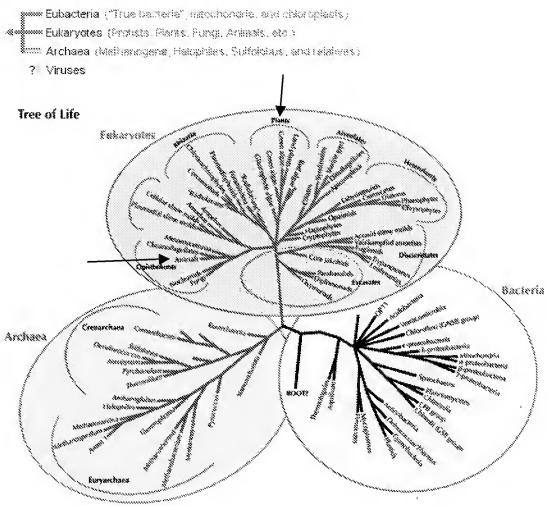
ordinary skill in the art to utilize the computational and experimental methods of Grad and Konings to predict and isolate miRNAs because of the evolutionary conserved miRNA identified by Lagos.

Applicant respectfully submits that consideration of multiple independent points demonstrate that the identification of viral miRNAs and hairpin precursors was not a predictable variation of the cited references and that one of ordinary skill in the art would not have had a reasonable expectation of success.

(1) One of ordinary skill in the art would not have expected viruses to contain miRNAs and hairpin precursors because miRNAs and hairpin precursors were believed to be present in only complex eukaryotes

The cited references disclose the identification of miRNAs and hairpin precursors only in complex eukaryotic organisms, such as vertebrate animals (humans, mice, rat), invertebrate animals (*C. elegans*, Drosophila), and plants. These organisms are highlighted on the phylogenic tree below from Barton *et al.*, Evolution, Cold Springs Harbor Laboratory Press (2007) and www.tolweb.org. As indicated by the arrows, the organisms that had been identified at the time of filing to contain miRNAs and hairpin precursors represent only two nodes of the eukaryotic domain, with no organisms being identified in the remaining three domains of life (bacteria, archibacteria, and viruses).

Phylogenetic trees are constructed by using genetic sequence analysis tools to show the predicted evolutionary relationships between various biological species. Within a phylogenic tree, the branch points and lengths of branches indicate the degree of genomic sequence similarity between different species. In other words, the farther apart two species are on a phylogenic tree, the more divergent the two species are at the level of genomic sequence. As can be seen below, viruses are separated from the three domained phylogenic tree of life indicating the divergence in sequence from eukaryotes, bacteria and archibacteria (*See* Barton *et al.*, Evolution, Cold Springs Harbor Laboratory Press (2007); www.tolweb.org)).



The cited references indicate that sequence conservation across species is a critical element for the identification of miRNAs and hairpin precursors using computational methods. Moss teaches that it is difficult to identify miRNAs using traditional biochemical methods.[5] Moss also teaches "[b]ioinformatics approaches are also currently limited."[6] Grad supports the difficulty of computational methods predicting miRNAs and hairpin precursors, as shown below:

[5] *See* Moss at pR138, column 2.

[6] *See* Moss at pR138, column 2.

> [Computational] methods take advantage of properties of known miRNAs, including their length …, precursor hairpin structure …, and tendency to be found in intergenic regions. However, the short length and high degree of sequence and structural variation also limit the accuracy of computational prediction based on sequence and structure alone.[7]

In order to overcome the difficulty in using computational methods to predict miRNAs and hairpin precursors, both Moss and Grad teach that sequence conservation should be taken into account. For example, Moss states:

> Lee and Ambros used conservation between *C. elegans* and its relative *C. briggsae* as an additional criterion, a strategy validated by the Bartel group's finding that 85% of the mir genes of C. elegans had recognizable homologs in *C. briggsae*.[8]

The use of sequence conservation is supported by Grad, which states:

> [W]e improved prediction methods by focusing on miRNAs that appear conserved across species or within a species. Our criterion of apparent conservation is "correspondence" – the presence in two or more genomes of short, very similar sequences embedded in the same stems of predicted hairpins with otherwise variable sequence.[9]

In discussing the teachings of the cited references, the Examiner clearly recognizes the importance of sequence conservation among species as a basis for identifying miRNAs and hairpin precursors. For example, the Examiner recognizes that Moss teaches "**phylogenically conserved** miR-1 precursor RNAs" in *C. elegans*, *Drosophilla* and humans.[10] The Examiner also states that the teachings of Lagos suggest that "many miRNAs are **evolutionary conserved**."

The sequence conservation of miRNAs and hairpin precursors is the focal point of the Examiner's rejection for obviousness. For example, the Examiner asserts that one of skill in the art would have implemented the algorithms and bioinformatics of the cited references "with a reasonable expectation success, because miRNAs were known to be **phylogenically universal**

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[7] *See* Grad at p1254, column 1.

[8] *See* Moss at pR139, column 1.

[9] *See* Grad at p1254, column 1.

[10] *See* Office Action at page 8 and *Moss* at Figure 1.

throughout various organisms."[11] Similarly, the Examiner asserts that it would have been obvious to one of skill in the art to explore the viral genome for miRNA precursors because "it was known that miRNA precursors are present in a **wide array of species and phylum**."[12]

Although the Examiner recognizes the importance of sequence conservation, the Examiner clearly misconstrues the diversity of known miRNAs and hairpin precursors. The cited references indicate that miRNAs and hairpin precursors had been identified only within a few small branches of the phylogenic tree - a limited number of complex eukaryotes such as vertebrate animals (humans, mice, rats), invertebrate animals (*C. elegans*, Drosophila), and plants. As shown in Appendix A, these organism represent only a small portion of the entire phylogenic tree across the four domains of life (eukaryotes, bacteria, archibacteria, and viruses). While the cited references disclose that computational methods may be useful to identify new miRNAs in the genomes of highly related eukaryotes, such as worms, flies, humans, and probably all animals, the cited references fail to state or demonstrates that the computational methods based on sequence conservation could be used to identify miRNAs and hairpin precursors in divergent single cell organisms (such as bacteria or yeast) or acellular organisms such as fungi, let alone viruses(See Moss at R138; Grad at 1254, and Lagos at Table 2).

Furthermore, it is clear at the time of filing that it was generally believed that miRNAs and miRNA precursors were not present outside of complex eukaryotes. For example, Lim *et al., Science* 299:1540 (2003)(hereinafter, "Lim") expresses doubt that miRNAs are present outside of complex eukaryotes. Lim reports an estimation of the number of miRNAs in the human genome based upon the methods of Moss, Grad and Lagos. Lim concludes the following:

> **There is no indication that miRNAs are present in single celled eukaryotes such as yeast**. It is tempting to speculate that the substantial expression of miRNA genes in plants and animals (and the apparent loss of miRNA genes in yeast) is related to their importance in specifying cell differentiation and development patterning.[13]

If Lim teaches that there is no indication of miRNAs in simple eukaryotic single cell organisms such as yeast, how would one of skill in the art expect miRNAs to be present in

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[11] *See* Office Action at page 13.

[12] *See* Office Action at page 14.

[13] *See* Lim at pg 1540, third column (with emphasis)

(i) other simple eukaryotic single cell species such as dinoflagellate (alveolata), ciliates (alveolata), brown algae (Phaeophycea), *Circogonia icosahedra* (Radiolaria-protozoa) or diatoms (Heterokontophyta); (ii) more primitive bacteria such as *Escherichia coli*, or *Stretococcus thermophilus*, (iii) ancient archaebacteria such as *Halophiles,* or (iv) the most remote domain of life – viruses?

Six months after the filing date of the instant application, viral miRNAs were identified in Pfeffer *et al., Science* 304:734-736 (2004) (*See* Pfeffer). Consideration of the circumstances surrounding the Pfeffer publication indicate that the consensus in the art at this time was that the identification of viral miRNA was a ground breaking new discovery (*See e.g.*, Science's commentary on Pfeffer *et al*; *Science* 304:645-648 (2004)). The Science commentary states the following:

> miRNAs had previously been found only in the genomes of plants and animals, each of which are complex eukaryotes. Pfeffer *et al* show the presence of miRNA in a virus, the '**fourth domain**' of life."[14]

The use of the words "fourth domain" indicates the appreciation that miRNAs had previously not been identified in this unrelated group and branch of the phylogenic tree of life.

Applicant respectfully submits that a proper review of the cited references in view of Lim and Pfeffer clearly indicates that one of ordinary skill in the art would have had tremendous doubts as to the presence of miRNA and hairpin precursors in viruses due to the critical lack of sequence conservation between viruses and the organisms previously identified to contain miRNAs and hairpin precursors.

The attached declaration of Dr. Alik Honigman (Appendix B) supports the position that one of ordinary skill in the art would not have had a reasonable expectation of success of identifying miRNAs and hairpin precursors in viruses. Dr. Honigman is an expert in the field of virology. Dr. Honigman states that there is little sequence conservation between the eurkaryotic, bacterial, archibacteria and viral branches of the phylogenic tree.[15] Due to this phylogenic divergence between the complex eukaryotic organisms known to harbor miRNAs at the time of filing (*i.e.*, humans, *C. elegans* and *Drosophila*) and more primitive organisms, Dr. Honigman

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[14] *See Science* at page 648 (emphasis added).

[15] *See* paragraph 3 of declaration.

states that he previously doubted whether viruses contained miRNAs.[16] In view of the foregoing, Applicant submits that those of ordinary skill in the art at the time of filing would not have had a reasonable expectation of success in identifying miRNAs in viruses because viruses were too divergent from the complex multicellular organisms known to harbor miRNAs.

(2) One of ordinary skill in the art would not have expected viruses to contain miRNAs and hairpin precursors because viral genomes are too small

At the time of filing, miRNAs and precursor hairpins were believed to be relatively scarce within the genome. This is important to consider because viral genomes are on average 10,000 times and up to a 1,000,000 times smaller than the size of the genomes from the organisms that miRNAs and hairpin precursors had previously been identified. The size of viral genomes are limited due to the size of the viral capsid.

Table 1 below lists the genome size for a number of organisms (column 2), as well as the number of hairpins precursors (column 3) reported in the Sanger database at the time of filing. The frequency of hairpins in each organism (column 4) can be calculated by dividing the genome size by the number of known hairpins. On average, the frequency of a hairpin precursor present in the genomes presented in the table below is at the rate of one hairpin precursor every 1.80×10^7 bp of the genome. As also shown below, the highest frequency of hairpin precursors is *C. elegans*, which is still, on average, only one hairpin precursor every 9.43×10^5 bases.

Table 1 – Frequency of Known Hairpin Precursors in the Genome

Organism	Genome Size (bp)	Known Hairpins	bp/Hairpin
Vertebrate			
Human	2.9×10^9	176	1.65×10^7
Rat	2.75×10^9	38	7.24×10^7
Mouse	2.64×10^9	202	1.31×10^7
Invertebrate			
C. elegans	1.0×10^8	106	9.43×10^5
Drosophila melanogaster	1.2×10^8	78	1.54×10^6
Plant			
Arabidopsis thaliana	1.57×10^8	43	3.65×10^6
Average			1.80×10^7

[16] *See* paragraph 3 of declaration.

By knowing the rate of known hairpin precursors, one can determine the expected number of hairpin precursors in other organisms by dividing the relevant genome size by the hairpin precursor frequency. Table 2 below lists the genome size for a number of viruses (column 2), which miRNAs and hairpin precursors had not been identified at the time of filing. Column 3 lists the number of hairpin precursors that would be expected for each organism using the average hairpin frequency shown above (1.80×10^7 bp). In each of the species of virus, Table 2 shows that less than one (1) hairpin precursor would have been expected to be present based on the average hairpin frequency in known organisms at the time of filing. Column 4 lists the number of hairpin precursors that would be expected for each organism using the highest hairpin frequency shown above in Table 1 (*i.e.*, *C. elegans*-9.43×10^5). Even at this highest hairpin frequency, Table 2 shows that less than one hairpin precursor would be expected for each viral organism.

Table 2 – Predicted Hairpin Precursors in Viruses

Organism	**Genome Size (bp)**	**Expected Hairpins-1 (average frequency)**	**Expected Hairpins-2 (highest frequency)**
Virus			
Epstein Barr Virus	1.75×10^5	0.0972	0.186
HCMV	2.30×10^5	0.0128	0.243
HPV	7.91×10^3	0.000439	0.00839

The above analysis shows that less than one hairpin precursor would have been expected to be present in a virus when using either the average hairpin or highest hairpin frequency as described above. Dr Honigman also states that he previously doubted whether the small genome of a virus would contain miRNAs and hairpin precursors.[17] Accordingly, Applicant respectfully submits that one of ordinary skill in the art would not have expected to be able to identify miRNAs and hairpin precursors in viruses regardless of the method of identification.

(3) One of ordinary skill in the art would not have expected viruses to contain miRNAs and hairpin precursors because viral genomes have less intergenic space

At the time of filing, miRNAs and precursor hairpins were believed to be found in the genome predominantly within intergenic sequences. This is important to consider because viral genomes contain on average 10,000-fold less and up to 1,000,000-fold less intergenic space than the genomes from the organisms that miRNAs and hairpin precursors had previously been

[17] *See* paragraph 4.

identified at the time of filing. Similar to the total size of the genomes, intergenic space in viruses is limited due to the size of the capsid.

Table 3 below once again lists the genome size for a number of organisms (column 2), as well as the number of hairpins precursors (column 4) reported in the Sanger database at the time of filing. Table 3 also lists the total amount of intergenic space in the genome of each organism (column 3). The frequency of hairpins in the intergenic space of each organism (column 4) can be calculated by dividing the size of the intergenic space by the number of known hairpins. As shown below, hairpin precursors are on average present at the rate of one hairpin precursor present every 1.21×10^7 bp of the intergenic space. As also shown below, even within the genome of the organism with the highest frequency of hairpin precursors (*C. elegans*) a hairpin precursor is present, on average, every 6.89×10^5 bp of the intergenic space.

Table 3 – Frequency of Known Hairpin Precursors in the Intergenic Space

Organism	Genome Size (bp)	Intergenic (bp)	Known Hairpins	Intergenic bp/Hairpin
Vertebrate				
Human	2.90×10^9	1.98×10^9	176	1.13×10^7
Rat	2.75×10^9	1.90×10^9	38	5.00×10^7
Mouse	2.64×10^9	1.70×10^9	202	8.42×10^6
Invertebrate				
C. elegans	1.00×10^8	7.30×10^7	106	6.89×10^5
Drosophila melanogaster	1.20×10^8	5.10×10^7	78	6.54×10^5
Plant				
Arabidopsis thaliana	1.57×10^8	6.00×10^7	43	1.40×10^6
Average				1.21×10^7

By knowing the rate of known hairpin precursors, one can determine the expected number of hairpin precursors in other organisms by dividing the relevant size of the intergenic space by the hairpin precursor frequency. Table 4 below once again lists the genome size for a number of organisms (column 2) that miRNAs and hairpin precursors had not been identified at the time of filing. For these same organisms, column 3 lists the size of the intergenic space. Column 4 lists the number of hairpin precursors that would be expected for each organism using the average hairpin frequency shown above (1.21×10^7 bp). In each of the species of virus, Table 4 shows that less than one (1) hairpin precursor would have been expected to be present based on the average hairpin frequency in known organisms.

Column 5 lists the number of hairpin precursors that would be expected for each viral organism using the highest hairpin frequency shown above (*C. elegans* at a frequency of 6.89 x 10^5). Even at the highest hairpin frequency, Table 4 shows that less one hairpin precursor would be expected for each viral organism.

Table 4 – Predicted Hairpin Precursors

Organism	**Genome Size (bp)**	**Intergenic (bp)**	**Expected Hairpins-1 (average frequency)**	**Expected Hairpins-2 (highest frequency)**
Virus				
Epstein Barr Virus	1.75×10^5	4.13×10^4	0.00341	0.063
HCMV	2.30×10^5	5.29×10^4	0.00437	0.0809
HPV	7.91×10^3	1.42×10^3	0.000653	0.00217

Similar to the previous analysis based on genome size, the above analysis shows that less than one hairpin precursor would have been expected to be present in a virus when using either the average hairpin or highest hairpin frequency as described above. Finally, Dr. Honigman also states that he previously doubted whether the small intergenic space of a virus would contain miRNAs and hairpin precursors.[18] Accordingly, Applicant respectfully submits that one of ordinary skill in the art would not have expected to be able to identify miRNAs and hairpin precursors in viruses regardless of the method of identification.

(4) Conclusion

The above remarks show a fatal divergence of sequence conservation between viruses and complex eukaryotes and doubts of the presence of viral microRNAs and hairpin precursors due to the small size of viral genomes and intergenic space. In view of each of these factual doubts about viral miRNAs and hairpin precursors, one of skill in the art at the time of filing would not reasonably have expected success in identifying viral miRNAs from the secondary structures of Yu/Konings using the methods of Moss, Grad or Lagos. In view of the foregoing, Applicant respectfully requests the Examiner reconsider and withdraw the rejection for obviousness.

[18] *See* paragraph 4.

c. 35 U.S.C. §112, 1st Paragraph, Written Description-Overbroad Genus

On pages 3-7, the Examiner rejects claims 21-49 under 35 U.S.C. § 112, first paragraph, for allegedly lacking written descriptive support on the grounds that the specification fails to support an overly broad claimed genus.

The Examiner alleges that the breadth of the claimed genus "viral nucleic acid" and subgenera "DNA virus," "RNA virus," and "retrovirus" embrace countless identified and unidentified nucleic acids derived from any genome and relies on Arauz-Ruiz *et al., J. of Gen. Virology* 83:2059-2073 (2002) and McGrath *et al, Virus Research* 76:137-160 (2001). Specifically, the Examiner asserts that Arauz-Ruiz teaches genetic variability of the different HBV serological types is due to the HBV surface antigen. With regard to McGrath, the Examiner asserts that like HBV, HIV and other retroviruses evolve through mutation and recombination. The Examiner concludes that specifically recited viruses (HBV and HIV for example) comprise genetically variable subtypes, which increase through mutation and recombination.

The Examiner's reliance on Arauz-Ruiz and McGrath to support the notion that there is divergence and diversity across various viral genuses/species/subtypes is misplaced as both references teach genetic variability in surface antigens: either the HBV surface antigen (See Arauz-Ruiz) or the *env* and *gag* coding sequences of HIV (McGrath).[19] Genes encoding viral surface antigens are well known to be extremely variable compared to other viral genes because surface antigens are a target for host immune responses. The Examiner fails to provide any connection between sequences encoding viral surface antigens and viral miRNAs. Accordingly, Applicant submits that there is no basis for the Examiner's allegation that there is improper written descriptive support for the claimed genus of viral hairpin/miRs based upon the sequence variability in viral surface antigens.

The Examiner further relies on *Fiddes v. Baird*, 30 USPQ2d 1481 (1985) as an example of a DNA patent application that did not convey with reasonable clarity to those skill in the art that the Applicant was in possession of the claimed invention. The application at issue in Fiddes had claims directed to all mammalian fibroblast growth factors (FGF), but was found unpatentable because the specification only provided a single FGF sequence isolated from

[19] McGrath teaches these mutations and recombination occur in HIV's *env* and *gag* coding sequences, which are the HIV capsid and surface ligand proteins (See McGrath at pg 143, second column, first full paragraph, pg 138, first column lines 10-14).

bovine. In stark contrast to the facts of Fiddes, the current specification clearly teaches a substantial number of viral hairpin/miRNAs with structural features. Specifically, the specification discloses and claims **1593 distinct viral hairpins** and **1797 distinct viral mature miRNAs**. These hairpin/miRNAs were identified from **85 distinct viruses** and constitute **26** DNA viruses, **55** RNA viruses, and **4** retroviruses (see Tables 1-3)[20]. Fiddes only discloses one bovine mammalian FGF sequence and tried to claim all mammalian FGF sequences. The Fiddes' facts regarding disclosure of one bovine FGF sequence to support claims to all mammalian FGF sequences is not remotely the same nor informative to compare to Applicant's disclosure of nearly 1600 hairpins and 1800 viral miRNAs across 85 distinct viruses. Therefore, the Examiner's comparison between Applicant's disclosure and *Fiddes* is misplaced for the purpose of establishing a representative number of the claimed invention, and in its place, Applicant submits that the disclosure of nearly 1600 examples of hairpins and nearly 1800 examples of miRNAs across 85 species of viruses provide an exhaustive level of written descriptive support of the structural criteria required of the claimed genus.

In summary, the Examiner's rejection relies on facts and case law that are not comparable to Applicant's claimed invention and written descriptive support for the claimed invention. One, the journal articles by Araus-Ruiz and McGrath cited by the Examiner focus on divergence of surface antigens to type viruses, but these facts provides no insight regarding whether there is substantial variation in viral miRNA sequences amongst various virus species. Second, the Examiner's reliance on Fiddes is not appropriate because a specification disclosing a single

[20] These viral hairpin/miRNAs also possess common attributes and features that are taught in the specification and indicated in the Table below.

Viral Structure	Viral Feature	Support
Viral Hairpin	Two Stem Segments that each are 19 to 71 nucleotides in length.	Page 4, line 29; and Table 2 in general; Example Table 2, lines 2030-2034; Table 2, lines 8569-8577
Viral Hairpin	Intervening Loop 3 to 19 nucleotides	Table 2 in general; Example Table 2, lines 37-31
Viral Hairpin	At least 44.1% complementation between first and second segments of hairpin	Table 2, ; Example, Table 2 lines 6086-6090
Viral Hairpin	-11.3 Kcal/mol	Table 2; Example Table 2 lines 6086-6090
Viral miRNA	17 to 24 nucleotides in length	Table 3, Example Table 3, line 1634; Paragraph 18
Viral miRNA	Binds trans related mRNAs	Page 4, line 29
Viral miRNA	Capable of inhibiting protein expression	Page 6, line 1
Viral miRNA	At least 72.7% complementation between miRNA and target mRNA	Table 4; Example Table 4, lines 350919-350925

bovine FGF sequence to support claims to mammalian FGF sequences is not remotely the same as the support provided by Applicant to the claimed invention. Applicant's disclosure of thousands of representative species allows one of skill in the art to recognize that all members of the claimed genus possess common attributes and features. In view of the foregoing, Applicant respectfully submits that the rejection of claims 21-49 under 35 U.S.C. §112, for allegedly failing to comply with the written description requirement due to an overly broad claimed genus has been overcome and a similar rejection of new claims 50 and 51 would be erroneous.

d. 35 U.S.C. 1st Paragraph, Written Description-New Matter

On page 6 and 7 of the Office Action, the Examiner variously rejects claims 21-49 under 35 U.S.C. §112, first paragraph for allegedly failing to comply with the written description requirement because the claims possess new matter not supported by the specification. Applicant respectfully disagrees.

Compliance with the written description requirement is essentially a fact-based inquiry that will necessarily vary depending on the nature of the invention claimed. An applicant may show possession in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting," such as by the disclosure of drawings, or structural chemical formulas that show that the invention was complete, or by describing distinguishing identification characteristics sufficient to show that the application was in possession of the claimed invention. See *Enzo Biochem. v. Gen-Probe, Inc.*, 323 F.3d 956, 963 (Fed. Cir. 1991) and *Regents of the Univ. of California v. Eli Lilly*, 119 F.3d at 1559, 1566 (Fed. Cir. 1997).

(a) Length Limitations 17 to 24 and 50 to 131 Supported by Specification

In the Office Action, the Examiner alleges that adequate support is lacking for the recitation of the miRNA length of 17 to 24 nucleotides and the hairpin length of 50 to 131 nucleotides. The Examiner finally asserts that although the SEQ ID NOS exemplified by Applicant may satisfy the structural limitations set forth in the claims, none of them satisfies the claimed length parameters (*i.e.*, miRNA lengths of 17 to 24 nucleotides, hairpin lengths of 50 to 131 nucleotides, hairpin stem segments of 19 to 71 nucleotides etc.).

With regard to the length limitations of the miRNAs (*i.e.*, 17 to 24 nucleotides) and hairpins (*i.e.*, 50 to 131 nucleotides) in the claimed invention, the Examiner has failed to consider the support and look at the data presented in the Tables. According to the M.P.E.P.'s

analysis of numerical range limitations, the Examiner must take into account which ranges one of skill in the art would consider inherently supported by discussion in the original disclosure (See 2163.05 III).

As discussed in our previous response and acknowledged by the Examiner, paragraph 18 of the specification teaches that the claimed viral miRNAs may be "about 18 to about 24 nucleotides in length." The specification further teaches two miRNAs that are 17 nucleotides in length. For example, SEQ ID NO: 2458 is a viral miRNA that is 17 nucleotides in length. This sequence is disclosed in Table 3, line 1634 and is shown below as in the previous response.

GENE NAME	VGAM SEQ-ID	GENE_SEQUENCE (5`_TO_3`)	PRECUR SEQ-ID	SOURCE REF-ID	SRC	VGAM ACC
VGAM2177.2	2458	GGGGGCTGAAGAAGGAC	84	NC_002512	100	

Similarly, the Examiner has acknowledged that paragraph 18 further teaches that the claimed hairpins may be "about 50 to 120 nucleotides in length." The specification also teaches 25 hairpins that are 131 nucleotides in length. For example, SEQ ID NO: 644 is a viral hairpin that is 131 nucleotides in length. This sequence is disclosed in Table 2, lines 253-261 and is shown below as in the previous response.

GENE NAME	PRECUR SEQ-ID	PRECURSOR-SEQUENCE (5`_TO_3`)	FOLDED-PRECURSOR	SRC	VGAM ACC
VGAM79	644	AATGTCAACCGCTTCAATGT GGCTATCACAAGGGCAAAAA TTGGCATTTTGTGCATAATG TCTGATAGAGATCTTTATGA CAAACTGCAATTTACAAGTC TAGAAATACCACGTCGCAAT GTGGCTACATT	CAA TTCA-- C CACAA AA-- G T G 101 C AATGT CCGC ATGTGG TAT GGGC AAATTG CA TTTGT CA TTACA GGTG TGCACC ATA TCTG TTTAAC GT AAACA GT TC- TAACGC - AAGA- AACA - C - T- **GATAG** TAA GTCT ATT TAGA TC -----		

GENE NAME	PRECUR SEQ-ID	PRECURSOR-SEQUENCE (5`_TO_3`)	FOLDED-PRECURSOR	SRC	VGAM ACC
VGAM66	639	AATAGAAGAGCTGCTGTCAT CTGTGCCTGCTTTGTATACA ATATGGGTATCTGGAGTTTC CAAATCCACATTTTCAGTAT ACAAAACAATTTCTGGAGCT AGGCCAGCAGCAGCCGCACT TCTGTT	A T CAT T - ---------- AAT-- 100 C AAG GC GCTGT CTG GCCT GCTT TGTATAC AT TTGTCTTC CG CGACG GAC CGGA CGAG ACATATG TA A C AC- - T GTCTTTAACAAA ACTTT - T C AGTTT G GG AT TGG C CC TA ACC A - A -----		

In view of the facts described above and that possession can be shown by Applicant in a variety of ways, the claimed viral miRNA length limitation of 17 to 24 nucleotides and the claimed viral hairpin length limitation of 50 to 131 is directly supported by the teachings of the

specification. Accordingly, Applicant respectfully submits that there is a sufficient description of miRNAs 17 to 24 nucleotides in length and hairpins 50 to 131 nucleotides in length to show one of skill that Applicant possessed the claimed invention at the time of filing.

(b) Length of Hairpin Arms and Loops Support by the Specification

In the Office Action, the Examiner further asserts that the specification fails to provide support for the claimed hairpin step segments of 19 to 71 nucleotides and loop segments of 3 to 19 nucleotides. The specification teaches on page 4, line 29 of the specification that viral nucleic acids are capable of forming hairpins.[21] With regard to the length of the hairpin arms (*i.e.*, 19-71 nucleotides) and loops (3-19 nucleotides), Applicant submits these lengths are described in the specification and can be determined by simply analyzing the 1593 disclosed viral hairpins disclosed in Table 2. As stated previously, Table 2 shows diagrams depicting stem-loop structures of the claimed nucleic acids. Analysis of the 1593 disclosed hairpins show that hairpin arm lengths range from 19 to 71 nucleotides. For example, Table 2, lines 2030-2034 discloses that the stem-loop structure for the nucleic acid as set forth in SEQ ID NO: 867 has a stem segment that is 19 nucleotides in length.

GENE NAME	PRECUR SEQ-ID	PRECURSOR-SEQUENCE (5'_TO_3')	FOLDED-PRECURSOR	SRC ACC	VGAM
VGAM677	867	CCATTAATATCTCTATTATA GGTTCTGGACATAATTCATC CCATTAAT TATTATACCAGAATTAATGG GGTAATTA	ATCTCTATTATAGCT AC **TCATCT** TCTGG ATAAT AGACC TATTA A-	100	

Table 2, lines 8569-8577 also discloses that the stem-loop structure for the nucleic acid as set forth in SEQ ID NO: 251 has a stem segment that is 71 nucleotides in length, as follows (the stem segment is in bold) and previously presented:

```
  CT  G      G      T    -    T ------     C-      ------------   A  C    TATCTT
TG  AG CCGCCT TTGTGG GGAT ACTG C       GCCCC  ACTGA             TCC TC ATTG
AC  TT GGTGGG GACACC CTTA TGAC G       CGGGG  TGACT             AGG AG TAAT
  TT  G      A      -    A    C CTTCCT     TC     TATTATGATGAA   -  T    ------
```

Analysis of Table 2 further shows 1593 hairpins with a loop size of 3-19 nucleotides. A statistical analysis of the disclosed hairpins indicates that over 98.1% of the hairpin loops are between 3 and 19 nucleotides in length.[22] For example, Table 2, lines 37-41 discloses that the

[21] Page 4, line 29 states "a nucleotide sequence of a first half of the RNA precursor is a partial inversed-reversed sequence of a nucleotide sequence of a second half thereof…"

[22] With a standard deviation of 3.2 nucleotides.

stem-loop structure for the nucleic acid as set forth in SEQ ID NO: 625 has a loop that is 3 nucleotides in length, as previously presented:

GENE NAME	PRECUR SEQ-ID	PRECURSOR-SEQUENCE (5'_TO_3')	FOLDED-PRECURSOR	SRC ACC	VGAM
VGAM8	625	AAACGCGGGCGTATTGGTCC CAATGGGGTCTCGGTGGGGT AAACGCGGG GT TTGGTCCCA ATCGACAGAGTGCCAGCCCT TTTGCGCCC CA AGCCAGGGT GGGACCGAACCCCGCGTTT	C A AT -- - G G **GTA** GG GGT CTC GT GG CC CCG GAG CA CT - - C- GA T A G ---	100	C

Table 2, lines 7507-7512 also discloses that the stem-loop structure for the nucleic acid as set forth in SEQ ID NO: 1302 has a loop that is 19 nucleotides in length, as previously presented:

GENE NAME	PRECUR SEQ-ID	PRECURSOR-SEQUENCE (5'_TO_3')	FOLDED-PRECURSOR	SRC ACC	VGAM
VGAM2526	1302	TATAATGTACTAGATAATAT TATAAAGCCTTTATTTAACC TATA TG ACTA AACCTATCTTCAAAGGACCG ATAT GC TGGT TCTGATGTTAAATGGTTCGA TATA	A T GA TAAAG- **ATTTAACCAACCTATCTTC** TAATATTA CCTTT ATTGTAGT GGAAA A T AA CTGCCA ----------	100	D

In view of the facts described above and that possession can be shown by Applicant in a variety of ways, the claimed hairpin stem length limitations of 19-71 nucleotides and viral hairpin loop sizes of 3 to 19 nucleotides are directly supported by the teachings of the specification. Accordingly, Applicant respectfully submits that there is a sufficient description of viral hairpins of a stem length of 19-71 nucleotides and viral hairpin loops of 3 to 19 nucleotides in length to show one of skill that Applicant possessed the claimed invention at the time of filing.

(c) Support for "At Least 44.1% Complementarity Between First/Second Stem Segments of Hairpin" and Free Energy of Hairpin is Found in the Specification

In the Office Action, the Examiner also asserts that there is no support for the claimed 44.1% complementarity between the first and second segments of a hairpin. The Examiner also asserts that the claimed value of –11.3 Kcal/mol for negative free energy of the hairpins is not specified or described in the specification.

The specification teaches in Table 2 that 1592 of the hairpins have at least 44.1% complementarity between the first and second stem segments of the hairpins. As previously discussed, Table 2, lines 6086-6090 discloses that the stem-loop structure for the nucleic acid as set forth in SEQ ID NO: 57 has a stem in which 44.1% of he nucleotides are complementary. The free energy of this hairpin with 44.1% complementarity is –11.3Kcal/mol.

GENE NAME	PRECUR SEQ-ID (5'_TO_3')	PRECURSOR-SEQUENCE	FOLDED-PRECURSOR	SRC VGAM ACC
VGAM2060	57	GGTGTGAATATCAAGCAGGA GAATATCAA- - GA- TAACAAG GATCT	CATAACAAGGTAGGATCTCT GGTGT GC AG CA GTAG ACAATACTTGGCACTAGCAG CCACA CG TC GT CATC CATTAATAACACC ATAATTACGA A ACG TCATAA- ----- -	101

With regard to negative free energy, the Examiner's allegation that Applicant is merely stating that the Matthews et al. reference satisfies the claimed value of –11.3 Kcal/mol is inaccurate. In Applicant's previous response, Applicant argued that Matthews describes a known method of calculating the free energy of a stem-loop structure. As stated above, the viral hairpin with the sequence as set forth in SEQ ID NO: 57 has a free energy of –11.3 Kcal/mol. One of skill would have been able to use the structure of SEQ ID NO: 57 shown in Table 2 as a basis for calculating a free energy of -11.3Kcal/mol. Matthews et al. is simply cited to reflect that it is possible to calculate the free energy of a hairpin given the structure of the hairpin. In other words, hairpin structures inherently have a free energy of folding and Matthews provides the formula for the free energy calculation. Accordingly, the structure of SEQ ID NO: 57 in the diagram shown in Table 2 discloses the free energy of –11.3 Kcal/mol and it is what it is, the free energy of the hairpin with the sequence as set forth in SEQ ID NO: 57.

In view of the facts described above and that possession can be shown by Applicant in a variety of ways, the claimed percent complementarity between the first and second stem segments of the hairpin and the free energy of the hairpin are supported by the teachings of the specification. Accordingly, Applicant respectfully submits that there is a sufficient description for the claimed percent complementarity between the first and second stem segments and the free energy of particular hairpins to show one of skill that Applicant possessed the claimed invention at the time of filing.

(d) Support for "At Least 72.7% Complementarity to Target mRNA" is Supported by the Specification

The Examiner also asserts that there is no support for the claimed 72.7% complementarity between a miRNA and target mRNA. The specification and Table 3 disclose that 1775 miRNAs are at least 72.7% complementary to a target mRNA. Support that one of the stems of the hairpin is a miRNA can be found at page 4, lines 29, which states "…RNA encoded by the bioinformatically detectable novel viral gene is about 18 to about 24 nucleotides in length, and originate from a RNA precursor…" The ability of a viral miRNA to act in trans and bind to an unrelated mRNA can be found at page 4, line 29, which states "…a nucleotide sequence of

the RNA encoded by a novel viral gene is a partial inverse-reversed sequence of a nucleotide sequence of a binding site associated with at least one host target gene..." The ability of the viral miRNA to inhibit the expression of a host protein can be found at page 6, line 1, which states "...the RNA encoded by the novel viral gene complementarily binds the binding site associated with at least one target host gene, thereby modulating expression of the at least one target host gene."

As previously discussed, Table 4 discloses that 1775 of the viral miRNAs have at least 72.7% complementary to a target mRNA. For example, Table 4, lines 350919-350925 shows that 16 out of 22 (72.7%) nucleotides of the miRNA as set forth in SEQ ID NO: 2964 are sufficient to bind the target gene LOC20314 as previously shown.

GENE NAME	VGAM SEQ-ID	TARGET	#BS	TARGET SEQ-ID	TARGET REF-ID	UTR	UTR OFFSET	TAR-BS-SEQ	BINDING-SITE-DRAW (UPPER:VGAM;LOWER:TARGET)	SRC	VGAM ACC	BS ACC	TAR ACC
VGAM934.1	2964	LOC2034 1	1	234992	XP_114697. 3	702		CAGTCT	CTTCGC	101	C	A	A
								GTCAGT	TTAATGTACA CTGACG				
								TACACT	GATTACATGT GACTGT				
								GTACAT	CACATT				
								TAGGGA					
								TA					

The application discloses 1797 viral miRNAs, which altogether have 590,161 target genes. An analysis of the amount of miRNA-target gene complementarity among the miRNAs and their targets reveals that 93% (±2.5%), or 1775 of the miRNAs have a target gene to which they bind with at least 72.7% complementation.

(e) Viruses in the dependents

The Examiner finally asserts that names of viruses introduce new matter by virtue of claim dependency. The Examiner has mistakenly misconstrued the claims. Independent claims 21 and 35 are directed to viral nucleic acids. Dependent claims 27-32 and 41-46 define the viral source of the nucleic acid. Applicant submits that the subject matter of claims 27-32 and 41-46 are supported throughout the specification and further define the type of viral nucleic acid of independent claims 21 and 35. Accordingly, Applicant respectfully submits that the names of viruses are not new matter in view of the claim language.

(f) Conclusion

As discussed above, each of the particular length limitations and percent complementation set forth for the miRNA and hairpins are further identifying characteristics that further describe the distinct viral genus of claimed nucleic acids. Applicant submits that the

Examiner has impermissibly focused her analysis of the characteristics only on each individual SEQ ID NO rather the entire specification, the data in the Tables, and all the SEQ ID NOS to determine if the particular length limitations or a percent complementation are described in the specification. In view of foregoing facts demonstration that possession can be shown by Applicant in a variety of ways, the claimed percent complementarity between claimed viral miRNA and a target gene mRNA is supported by the teachings of the specification. Accordingly, Applicant respectfully submits that there is a sufficient description to show one of skill that Applicant possessed the claimed invention at the time of filing. In view of the foregoing remarks, Applicant respectfully submits that the rejection of claims 26-41 under 35 U.S.C. § 112, first paragraph, has been overcome and should be withdrawn.

e. Nonstatutory Obviousness-Type Double Patenting

On pages 15-18 of the Office Action, the Examiner rejects claims 21-23, 25, 26, 29, 31-37, 39-40, 43, and 45-49 on the ground of nonstatutory obviousness-type double patenting as allegedly being unpatentable over claims 1-5 of U.S. Pat. No. 7,217,807. The Examiner also provisionally rejects claims 21-23, 25-28, 35-37, and 39-42 on the ground of nonstatutory obviousness-type double patenting as allegedly being unpatentable over claims 21-23 and 32-36 of copending App. No. 10/707,003. The Examiner further provisionally rejects claims 1-23, 25-37, and 39-49 on the ground of nonstatutory obviousness-type double patenting as allegedly being unpatentable over claims 1-8 and 12 of copending App. No. 10/605,838.

Applicant respectfully requests that the Examiner hold the rejection in abeyance until there is allowable subject matter, at which time the Applicant will consider amending the claims in U.S. Pat. App. Nos. 10/707,003 or 10/605,838, or filing a terminal disclaimer.

3. Conclusion

Applicant respectfully submits that the instant application is in good and proper order for allowance and early notification to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite prosecution of the instant application, the Examiner is encouraged to call the undersigned at the number listed below.

Respectfully submitted,

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